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BIOLOGICAL RHYTHMS IN PEROGNATHUS

by

R. G. Lindberg, R. M. Chew, and P. Hayden

Introduction

Biological rhythms are recognized as a fundamental characteristic of living systems. Since their formal recognition by De Mairan in 1729 to the present, the question as to whether these rhythms are set by endogenous or exogenous cues or reflect the interaction of the two has presented a formidable challenge to the experimental biologist. Recent advances in space technology now permit the placing of organisms demonstrating pronounced rhythms into earth orbits thereby promising to elucidate the question of the influence of exogenous cues (Zeitgeber) on biological rhythms.

Pocket mice (Genus Perognathus) were proposed by NSL as particularly suitable subject matter for space biology research by virtue of their unusual physiology which in turn allowed significant compromises in the life support requirements for mammals in biosatellites. The resultant savings in payload weight coupled with the small size of the animals were anticipated to permit experimental designs with statistically significant numbers of animals and good reliability. This contract (NASr-91) was awarded to test these assumptions and to design a definitive "space" experiment utilizing the unusual characteristics of the pocket mice.

In the course of establishing metabolic base lines for the pocket mice (Chew and Hayden, 1962), numerous data have been obtained which strongly suggest the presence of a biological rhythm in P. longimembris which is about 24 hours long (circadian). The intent of this paper is to present those data which indicate the existence of a circadian rhythm preparatory to the preliminary design of an experiment to test the effect of extra-terrestrial residence upon that rhythm.

It should be emphasized that the research performed to date was not designed to study or test circadian rhythms and as a result obvious gaps in the experimental data exist. It is significant, however, that despite variations in food supply,

[redacted]

ambient temperature, atmospheric composition, photoperiod, and gamma-irradiation stress, a biological rhythm has persisted.

Methods

For a description of methods and materials concerning the pocket mouse study, refer to Chew and Hayden (1962). Unless otherwise noted, the animals were kept individually in 1-liter bottles and oxygen utilization was monitored.

Discussion

Circadian rhythms have been shown for numerous rodents and other mammals under normal environmental conditions as measured with activity wheels (Halberg, 1959; Rawson, 1960), body temperature (Hellbrugger, 1960), and rate of metabolism (Pearson, 1960).

Perognathus is unusual in the depth to which its metabolism can drop during its resting periods. Even when food is available and environmental temperatures are moderate, pocket mice frequently allow their rate of metabolism to drop below the minimum needed to maintain a normal mammalian body temperature as in Fig. 1. (This is referred to as hypometabolism in the present report.) When pocket mice are starved and temperatures are low, they allow their metabolic rate to drop every day to near a level equal that found in hibernating mammals. (This is referred to as deep hypometabolism in the present report.) At such times, a mouse's internal temperature approximates that of his environment.

It has been known for a long time that similar daily metabolic fluctuations occur in bats (Hock, 1951), and more recently there have been reports for the Birch Mouse (Johansen and Krog, 1959).

The factors responsible for circadian rhythms in mammals are only partly understood, especially the endogenous and subtle exogenous factors. This topic is considered in Cold Spring Harbor Symposium [25(1959)] on "Biological Clocks" which summarizes much of the literature. The following examples indicate the existence of a circadian rhythm in pocket mice as evidenced by measurement of oxygen utilization.

I. Pocket mice under moderate normal environmental conditions.

Most *P. longimembris*, when they are kept individually in 1-liter beakers at a moderate temperature (22-24°C), show obvious alternating periods of high and low oxygen consumption (see individual A in Fig. 1). Even when food is provided, some individuals become hypometabolic (see individual B in Fig. 1). At moderate temperatures and with food present, deep hypometabolism occurs with a low incidence (see Groups 1 and 2 in Table I).

II. Effect of factors experimentally varied from normal.

A. Withdrawal of food at 22°C ambient (see Group 3 in Table I). Starvation accentuates the amplitude of the metabolic rhythm, with animals becoming deeply hypometabolic every day (see Fig. 2). There is not an exact coincidence of low points of individual animals at moderate temperatures, but there is general agreement as to the lengths of the cycles of individual mice as shown in the integrating bar graph, Fig. 3.

The incidence of torpidity (the number of hypometabolic periods on which the animal is deeply hypometabolic) in the Little Pocket Mouse when starved at moderate temperatures may be 100% (Group 3, Table I).

B. Withdrawal of food at 10°C ambient (Group 4, Table I). Individually housed mice starved at 10°C showed an accentuated rhythm, as at 22°C. There was somewhat greater coincidence of times of midpoints of deep hypometabolism (Fig. 3), compared to the 22°C group, and the periods of deep hypometabolism are much more prolonged. (Compare dark bars in Fig. 3.) Several mice remained deeply hypometabolic for two or three days in succession, but this is unusual in mice kept isolated from other individuals.

Grouped individuals under the same conditions demonstrate a group metabolic rhythm, as in Fig. 4. Here the majority of animals show a 24-hour rhythm beginning with the metabolic low on the second day. A secondary 24-hour rhythm, which begins late in the third day, may represent one or more animals that are "out of step."

C. Animals in darkness. (Groups 6 and 7 in Table I) The metabolic rhythm of groups of *P. longimembris* (Group 6, Table I) and also *P. inornatus*

(Group 7, Table I; Fig. 5) persists in continuous darkness, at least for 7 days.

Periodic arousals are not eliminated by subjecting the animals to an atmosphere which is 4.5% carbon dioxide (Group 6, Table I).

Two mice were kept individually in a dark, sound proof box for 11 days. One animal showed an unusually precise 24-hour rhythm, the other showed an unusual, but precise, 48-hour rhythm (Fig. 6).

D. Effect of acute radiation (Group 8, Table I.). P. longimembris is able to survive radiation dosages that are fatal to other mammals (Gambino and Lindberg, 1963). Nine mice, which had been subjected to 1400 roentgen dosage, were shortly thereafter placed within a normal environment for metabolic measurements (Fig. 7). Metabolic rhythms were demonstrated in all individuals. The rhythm differed from controls only in a greater incidence of periods of deep hypometabolism (compare Groups 2 and 8 in Table I).

Some of the irradiated animals had a hypometabolic sequence that is very similar to the food deprived groups (compare Fig. 7 and Fig. 2); however, most of this group, after an initial loss of 1-2 g during the first week, gained weight during the second week of the study.

The irradiated animals were disturbed on the seventh day for weighing and examination. After this disturbance there was an average shift of 4.3 hours (6.0 - 0.3) of the midpoint of deep hypometabolism towards later in the day. No such shift was observed in non-irradiated animals disturbed in the same way (Table I, Group 2). Six animals were restudied 79-83 days after irradiation. The rhythms noted 1-14 day following irradiation tended to persist.

III. Comparison of metabolic behavior of isolated and grouped pocket mice.

A. The data suggest that when pocket mice are kept in a group within the same chamber or in a series of chambers on the same air flow there is a synchronization of the metabolic rhythms of individuals.

While there is an obvious group rhythm in grouped individuals (Fig. 4 and 5), there is not an equivalent synchronization of individuals that are measured separately at the same time. (This may be a case of social entrainment of rhythms.)

B. The data suggest that when animals are kept in groups, there is a much greater tendency for individuals to become deeply hypometabolic and remain so for several days. In Fig. 4 the group as a whole was hypometabolic from early in the third day onward. Similarly, in Fig. 5 the group was hypometabolic from the fourth day onward.

It is concluded that the "group" hypometabolism is the result of one or more individuals failing to arouse at the expected time. The "group" rhythm persists because of those animals that do arouse, to create the peaks in the curve.

Summary and Conclusions

1. Pocket mice (*P. longimembris* and *P. inornatus*) demonstrate a circadian metabolic rhythm.

2. This rhythm is obviously in agreement with the natural photoperiod; the mice, which are nocturnally active under natural conditions, show metabolic lows during daylight hours and metabolic highs during nighttime hours. This occurs even when animals are in continuous darkness for 6-7 days.

3. Starvation and low ambient temperatures accentuate the amplitude of the rhythm so that there is an occurrence of deep hypometabolism almost every day.

4. The approximate 24-hour period of the rhythm is not altered by keeping individual pocket mice in continuous darkness, isolating them from sound, exposing them to air with 4.5% CO_2 or to atmospheres saturated with water vapor, or exposing them to 1400 r Co^{60} irradiation.

5. When starved mice are kept in groups, there is a tendency for their rhythms to become synchronized, and also for some individuals to "drop out of the rhythm" for one or more days by remaining deeply hypometabolic.

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TABLE I

Group and Conditions	Arrangement of Animals	Midpoint of Metabolic Lows	Interval Between Metabolic Lows	Incidence of Deep Hypometabolism/ Hypometabolism
(1) 24°C, Food, Day-Night, Dry 100% O_2 September 30 - October 4, 1962	7 Mice, Individually housed and monitored	1405 hrs.(1100-1515)	23.9(22.5-25.5)hrs	3/27 or 10.7%
(2) 22°C, Food, Day-Night, Dry 100% O_2 April 30 - May 14, 1963	9 Mice, Individually housed and monitored	1148 hrs.(0430-1736)	24.8(13.0-30.0)	35/114 30.7%
(3) 22°C, Starved, Day-Night, Dry 100% O_2, May 21-May 25, 1963	7 Mice, Individually housed and monitored	0800 (0100-1600)	24.5 (21.0-28.5)	21/21 100 %
(4) 10°C, Starved, Day-Night, 100% O_2 Saturated with water vapor February 19 - February 26,1963	5 Mice, Individually housed and monitored	0636 (0318-1000)	23.1 (20.7 - 25.2)	30/30 100 %
(5) 10°C, Starved, Day-Night, Dry 100% O_2, March 19 - March 26, "63	6 Mice in one chamber, monitored together	1033 (0830 - 1300)	25.2 (23.5 - 28.5)	6/7 85.7%
(6) 20°C, Starved, Dark, Dry Air with 45% CO_2, nest material [1]. January 4 - January 9, 1963	6 Mice in series of chambers: monitored together	0700 (0245 - 1325) [1823 (1445 - 2300)][2]	22.7 (19.0 - 26.8)	100 %
(7) <u>Perograthus inornatus</u>[3], 10°C, Starved, Dark, Air saturated with water vapor, nest material, February 20 - February 26, 1963	6 Mice in series of chambers: monitored together	0640 (0500 - 0800)	23.2 (21.0 - 25.0)	100 %
(8) 22°C, Food, Day-Night, Dry 100% O_2, after 1400 roentgen exposure March 25 - April 8, 1963	9 Mice, Individually housed and monitored	0902 (0718 - 1018)[4] 1232 (0736 - 1518)[4]	24.2 (20.0 - 27.0)[4] 23.8 (18.0 - 27.0)[4]	51/96 53.1%

(1) Nesting material not provided unless specified; animals kept over granulated absorbent,"Drizit."

(2) Midpoints of metabolic highs, which could be read more accurately than lows in this case

(3) All other data for <u>P. longimembris</u>. These data from Chew (1963)

(4) Values for first and second weeks of two week period. Disturbed at 7 days for weighing.

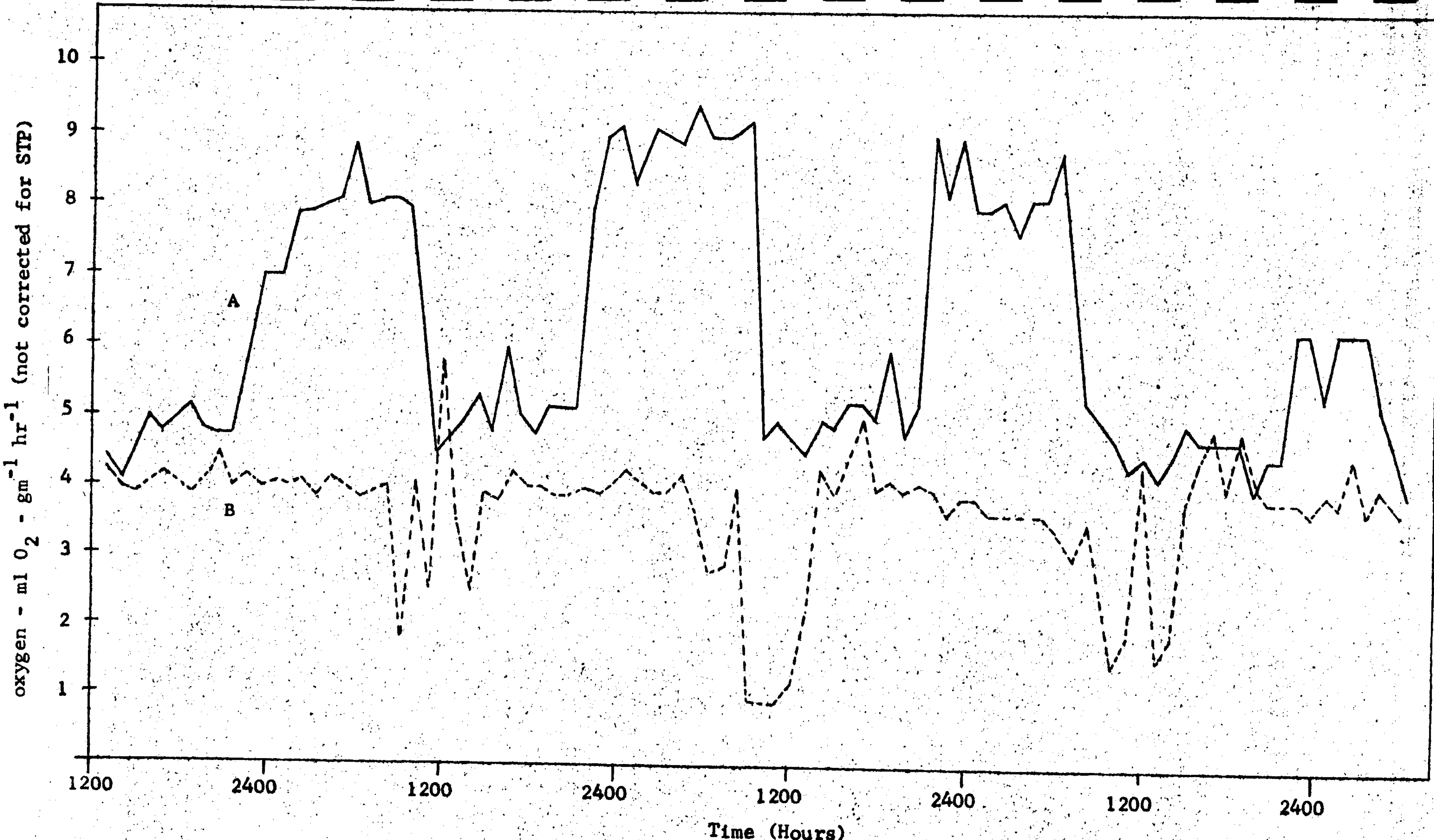

Figure 1. Metabolic activity of 2 representative P. longimembris maintained at 24°C with food (Table I, Group 1).

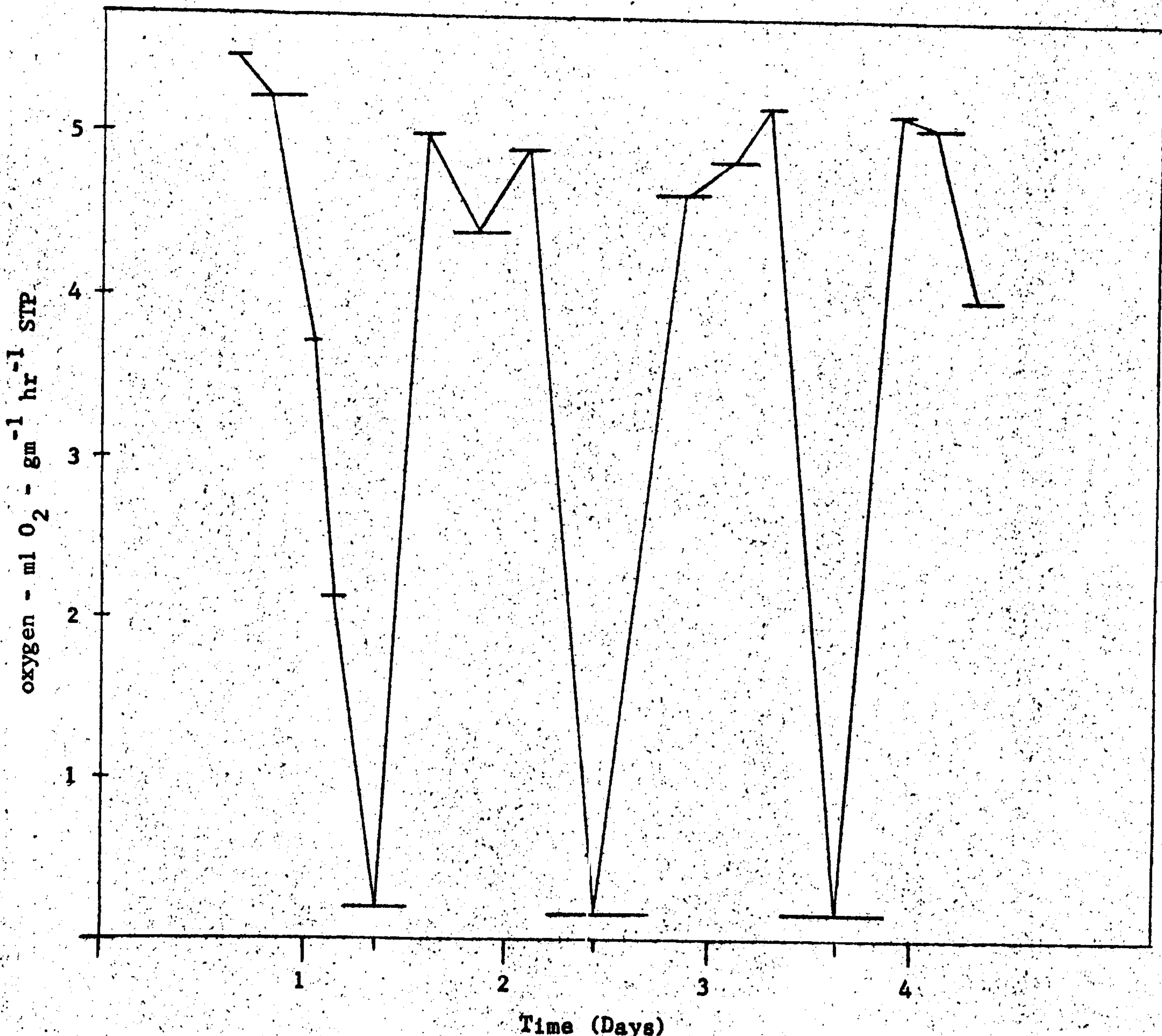

Figure 2. Metabolic activity of a single P. longimembris maintained at 22°C with no food (Table I, Group 3).

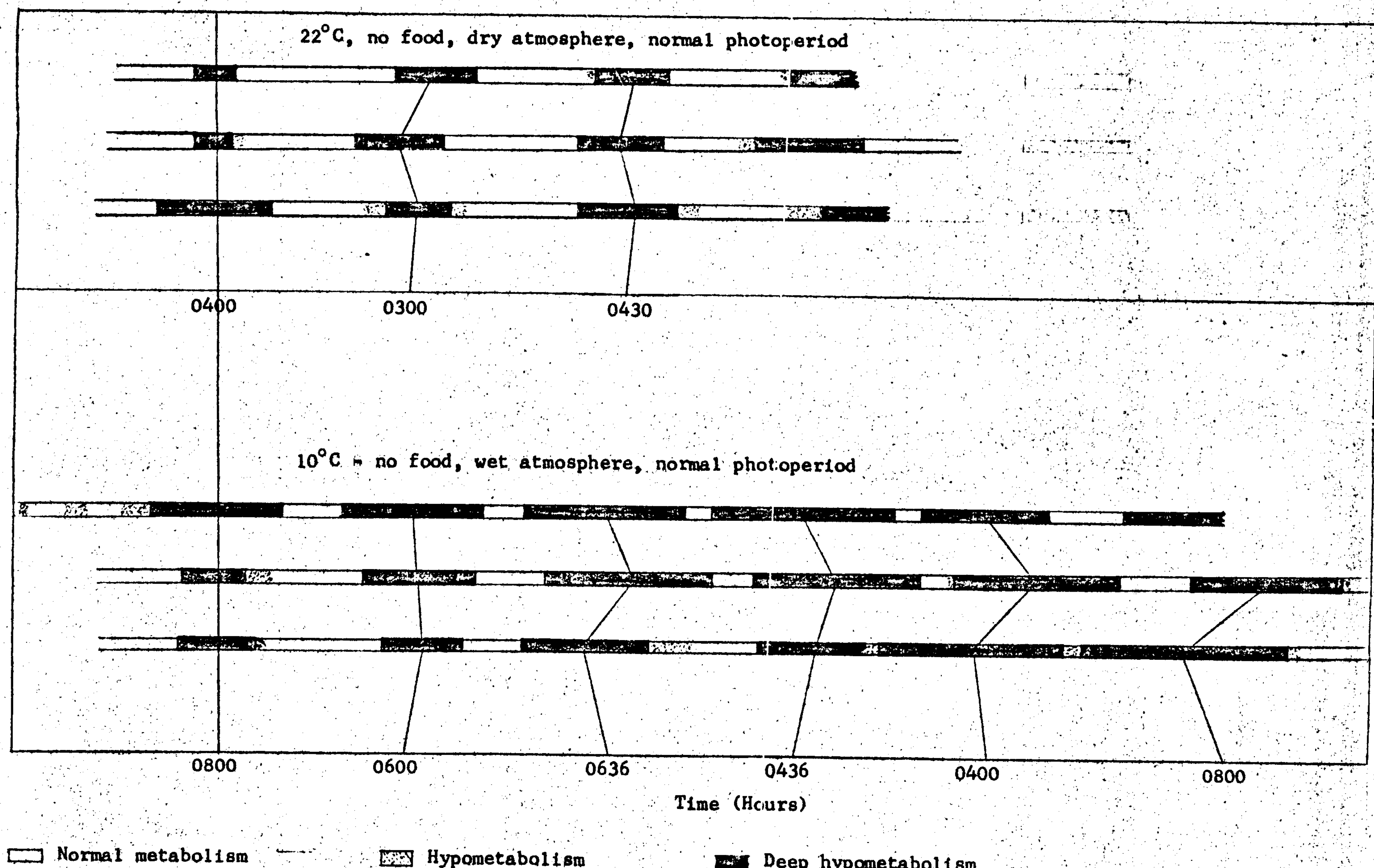

Figure 3. Comparison of rhythm of hypothermic periods in two groups of three P. longimembris studied under different environmental conditions.

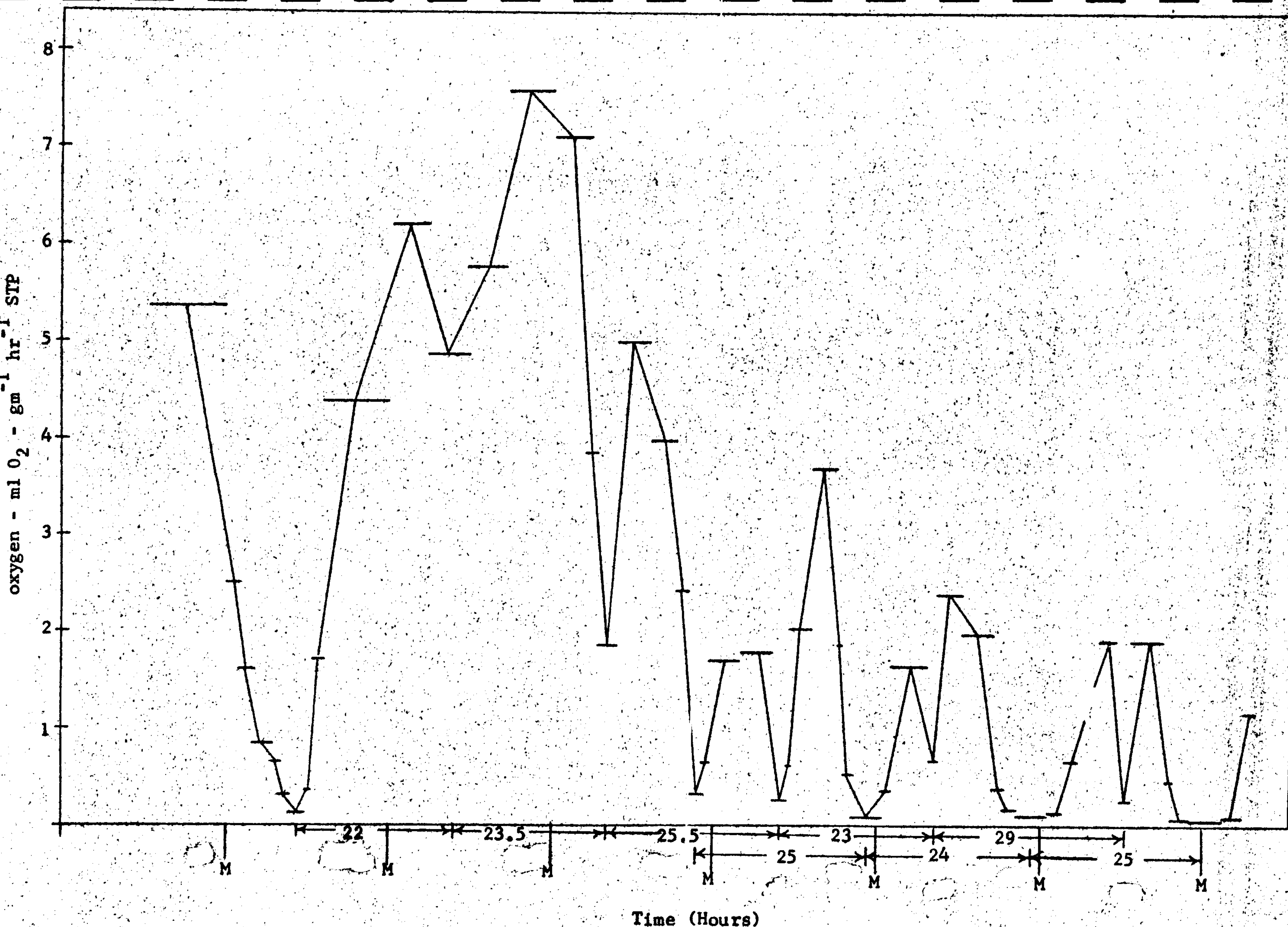

Figure 4. Metabolic activity of 6 <u>P. longimembris</u> monitored together, normal photoperiod at 10°C (Table I, Group 5), no food.

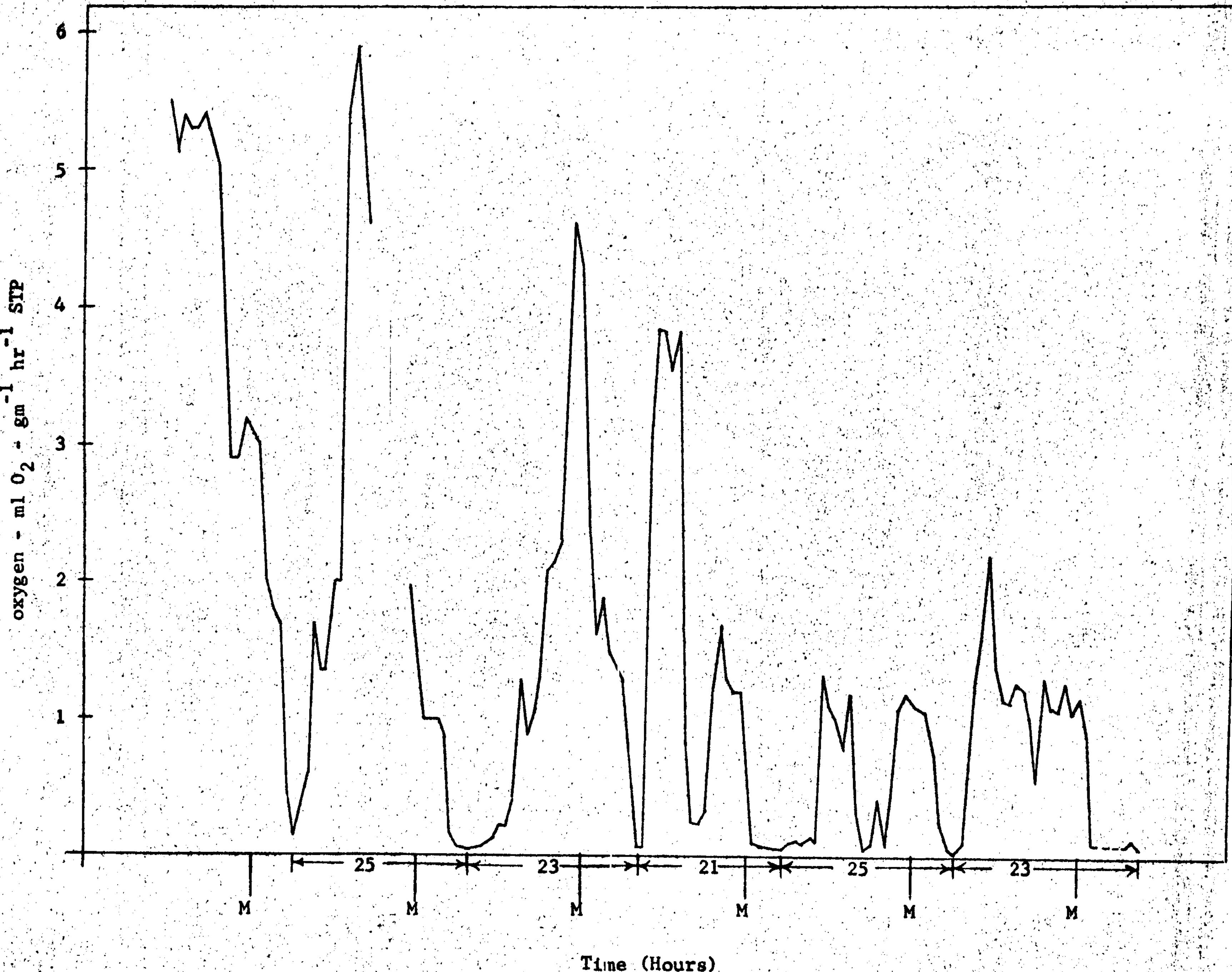

Figure 5. Metabolic activity of 6 P. inornatus monitored together in dark chamber at 10°C (Table I, Group 7), no food.

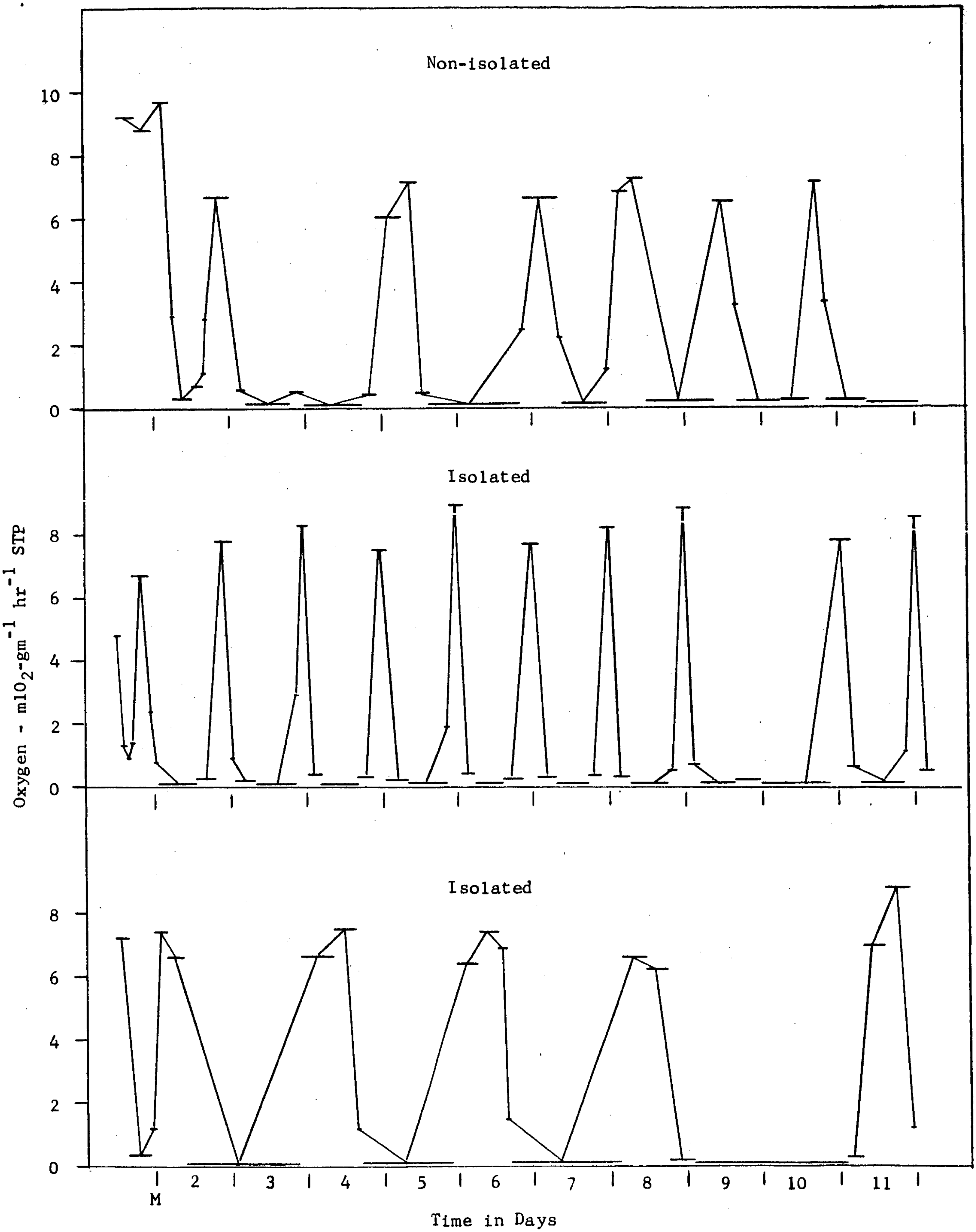

Figure 6. Relative metabolic activity of 3 P. longimembris in "isolated" and "non-isolated" chambers.

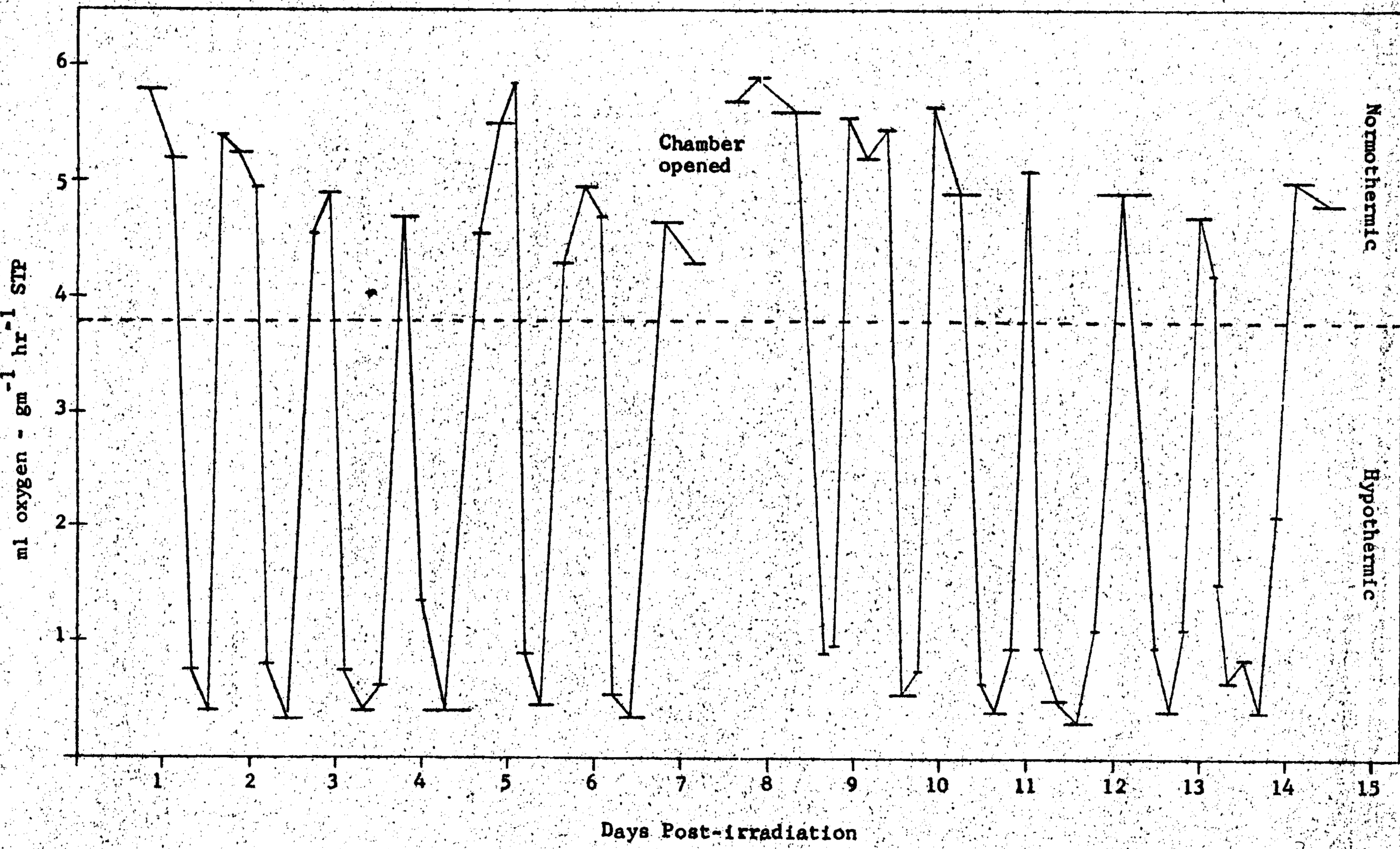

Figure 7. Metabolic activity in P. longimembris (♀) following 1400 r acute whole body cobalt^{60} irradiation (animal at 22°C with food) (Table I, Group 8).

APPENDIX I

PRELIMINARY DESIGN FOR A

BIOLOGICAL EXPERIMENT IN SPACE

PRINCIPAL INVESTIGATOR: Robert G. Lindberg and Robert M. Chew

INSTITUTION: Northrop Space Laboratories and Univ. of Southern California

ADDRESS: Hawthorne, California - Phone: ORegon 8-9111

Experiment Title:

The effect of extra-terrestrial residence on the circadian metabolic rhythm of pocket mice (_Perognathus longimembris_).

Working Concepts:

Data from metabolic studies on pocket mice strongly suggest that _Perognathus longimembris_ has a circadian metabolic rhythm which can be detected at both moderate (22-24°C) and low (10°C) environmental temperatures, at low humidities (less than 10% relative humidity) and saturated air, in the dark or under normal photoperiod, with and without food, in normal atmospheres and 100% oxygen, and in both individually housed and in grouped mice (see NSL 62-125-4). It is anticipated that placing these animals in earth orbit will elucidate the effects of exogenous factors which may influence what appears to be a dominant endogenous rhythm. While the most obvious exogenous cue to be studied is weightlessness, the experimental design is easily adaptable to provide for the input of almost any specific environmental stimuli in the isolation of space.

Experimental Design:

Four units of 8 mice each (P. longimembris) to be subjected to a minimum of two weeks in orbit. At least the first week to be in a weightless condition with artificial gravitational force provided the second week if anomalies in the rhythms become apparent. Since availability of food is known to influence the depth of hypometabolism, the following feeding schedule will be maintained.

Unit one: Fed both first and second weeks.

Unit two and three: Not fed first week, fed second week.

Unit four: Fed first week, not fed second week.

Oxygen consumption of each unit to be monitored in flight with data readout adapted for computer analysis on ground. If circadian rhythm not present first week, and first unit with normal metabolic level, artificial gravity to be introduced second week in form of centrifugal force developed by spinning either vehicle or experimental package.

Potential Conclusions:

(1) If units one and four maintain normal metabolic rate, with or without rhythm, during the first week, it is demonstrated that weightless animals can feed adequately, and physiological function must be proceeding relatively normal.

(2) If there is no circadian rhythm the first week, and animals are feeding, and if rhythm is regained during second week (under artificial gravity), this is evidence that gravity is clue for metabolic rhythm.

(3) If circadian rhythm persists in weightless condition and animals are feeding, gravity is not an important clue to setting of rhythm.

(4) If failure of feeding, and no rhythm, either under weightlessness or artificial G, then physiological functions grossly interfered with and may or may not be linked to weightless exposure.

General Experimental Requirements

1. Lead time: Weeks
2. Time:
   a. Prelaunch hold time: Not critical up to 48 hours.
   b. Orbit time: Minimum 14 days.
   c. Maximum post-flight time: Recovery unnecessary if telemetry available.
3. Radiation exposure: Not to exceed 1400 r over 14 days.
4. Environmental conditions:
   a. Temperature: Prelaunch 10° to 35°C; Flight 8-12°C; Recovery 10° to 35°C.
   b. Atmospheric composition: Depends upon how metabolic activity is to be monitored, e.g.,

      100% oxygen, if consumption monitored in terms of decreasing pressure;

      ~~approx.~~ 21%, if consumption to be monitored in terms of decreasing oxygen concentration;

      either composition if CO_2 production is measured in lieu of O_2.

      Atmospheric pressure: ~15 psi
   c. Other:

      Animals in dark.

      Humidity to be constant, level not critical, but preferably low (<30%).

      Device for imparting spin to animal chambers or vehicle during second week to be used if desired, depending upon results of first week.

      Means controlling ammonia.
5. Operational constraints imposed by experiment:
   a. Maximum permissible acceleration: Unknown
   b. Maximum permissible spin at launch and reentry: Unknown
   c. Does slow rotation on tumbling effect experiment? Yes
   d. Other:
6. Weight estimates: Minimum of 32 mice, 9.5 grams each -

   <u>Unit one</u>: Assuming mice will remain normothermic and normally metabolic as on earth, at rate of 9 ml O_2/g hr, respiratory quotient of 0.75, caloric equivalent of oxygen of 4.8 cal/ml, food of 6 Kcal/g caloric value, evaporate at rate 1 mg/ml O_2.

One mouse: (9.5 g) (9 mlO_2/g hr) (24 hrs) (14 days) =
28.7 liters O_2 (40.2 g) used while consuming 23 grams of seed (sunflower) producing 0.75 X 28.7 = 21.5 l CO_2 or 42.1 g and losing 28.7 g water (at rate 1 mg H_2O per ml O_2).

Vehicle must provide 8 X 40.2 gm oxygen, 8 X 23 grams sunflower seed, absorbent for 8 X 42.1 gm CO_2, absorbent for 8 X 28.7 gm water.

Units two, three and four: These are to be provided with four days of food for each mouse, to be issued either at start or at beginning of second week. Animal must be provided enough oxygen to utilize this food and 50% of its own body weight. (If animals do not become torpid, and thus stay within this nutrient supply, they will have starved to death before exceeding this O_2 supply.)

To use up $\frac{(6.6 \text{ gm food})\ (6 \text{ Kcal/gm})}{4.8 \text{ Kcal/liter}}$ = 8.25 liters O_2 or 11.55 gm O_2.

To use up 4.75 gm body weight, or 1.9 gm body solids, with max. caloric value of 9 Kcal/gm.

Units two, three and four require, for total of 24 mice

24 X (11.55 + 5.0) gm oxygen - 397 gm oxygen

24 X 11.81 X 0.75 X 1.67 = 355 gm CO_2 absorbence

24 X 11.81 = 284 gm water absorbence

24 X 6.6 = 156 gm food as sunflower seed

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Results of investigations of biological rhythms in pocket mice have led to the following conclusions: (1) Pocket mice (*Perognathus longimembris* and *Perognathus ignoratus*) demonstrate a circadian metabolic rhythm. (2) This rhythm is obviously in agreement with the natural Photoperiod; the mice, which are nocturnally active under natural conditions, show metabolic lows during daylight hours and metabolic highs during nighttime hours, even when exposed to continuous darkness for 6 to 7 days. (3) Starvation and low ambient temperatures accentuate the amplitude of the rhythm so that there is an occurrence of deep hypometabolism almost every day. (4)The approximate 24-hour period of the rhythm is not altered by keeping individual pocket in continuous darkness, isolating them from sound, exposing them to air with 4.5% CO_2 or to ~~a~~ atmospheres saturated with water vapor, or exposing them 1400 r Co^{60} irradiation. (5) When starved mice are kept in groups, there is a tendency for their rhythms to become synchronized, and forsome individuals to "drop out of the rhythm" for one or more days by remaining deeply hypometabolic. These conclusions have led to the preliminary design of a biological experiment in space for studying the effect of extraterrestrial residence on the circadian metabolic rhythmof pocket mice (*Perognathus longimembris*). I.v.L.

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ABSTRACT

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